3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN ER BINDING ASSAYS

3.1 Introduction

ER binding data were obtained for a total of 638 substances **(Appendix C)**. While a relatively large number of substances have been tested in ER binding assays, only a small number of these substances were evaluated in multiple types of ER binding assays and/or by multiple laboratories. With the exception of 17β-estradiol, the reference estrogen used in most studies, only 14 substances (2.2%) were tested in at least 10 of the 14 assays considered in this BRD. These substances are bisphenol A, 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), kepone, coumestrol, *o,p'*-dichlorodiphenyltrichloroethane (*o,p'*-DDT), diethylstilbestrol (DES), 5α-dihydrotestosterone, estriol, estrone, 4-hydroxytamoxifen, genistein, methoxychlor, tamoxifen, and zearalenone. Ninety-four percent (600) of the substances in the database were tested in five or fewer assays, 63% (403) were tested in one assay only, and 59% (376) were tested in one publication only.

3.2 Rationale for Selection of Substances/Products Tested in *In Vitro* ER Binding Assays

Most of the substances tested in *in vitro* ER binding assays closely parallel the initial studies on the isolation and characterization of the receptor, the subsequent synthesis and characterization of ER agonists and antagonists, and the more recent use of ER binding assays as a method for endocrine disruptor screening. Many of the first substances to be tested were selected to address basic research questions regarding the nature of the ER and the kinetics of its interactions. A number of the triphenylethylenes, stilbenes, and DES analogs and derivatives, for example, were investigated to obtain a better understanding of ER binding processes. Some substances were investigated in research and development studies designed to determine which metabolite or stereoisomer of a molecule enhanced or inhibited binding to the ER. Data from these types of studies often contributed to the development of pharmaceuticals for breast cancer, estrogen-replacement therapy, or for other health concerns. Some substances were investigated to determine structure-activity relationships (SAR) for the development of quantitative SAR (QSAR) models. Finally, during the last decade, with the growing concern about possible adverse health effects associated with exposure to endocrine disruptors, some of these substances (e.g., pesticides, polychlorinated biphenyls, phytoestrogens) were tested using *in vitro* ER

binding assays to identify those that may act as estrogen agonists/antagonists in humans and wildlife.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in ER binding assays is provided in **Appendix C**. Substances were assigned to a single chemical class based on available information from standardized references (e.g., *The Merck Index 12th Edition* and the U.S. National Library of Medicine's ChemID database) and from an assessment of chemical structure. As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* ER binding data are polychlorinated biphenyls, phenolic and nonphenolic steroids, triphenylethylenes, organochlorines, polycyclic aromatic hydrocarbons, stilbenes, phenols, and bisphenols. Of the 638 substances included in **Appendix C**, seven substances were not classified within a chemical class.

Product classes were assigned based on information contained in *The Merck Index* and the U.S. National Library of Medicine's ChemFinder. As show in **Table 3-2**, the most common product classes tested in *in vitro* ER binding assays have been pharmaceuticals, pesticides, chemical intermediates, dielectric fluids or their components, natural products (including several phytoestrogens), and plasticizers. Of the 638 substances included in **Appendix C**, 320 were not classified within a product class.

Table 3-1 Chemical Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Chemical Class	# of Substances
Acetamide	2
Acrylate	6
Alcohol	4
Aldehyde	1
Alkoxyphenol	5
Alkylbenzene	2
Alkylphenol	14
Amide	1
Anilide	2
Aniline	4
Aromatic amine	1
Aromatic heterocycle	1
Aromatic hydrocarbon	1
Azo compound	1
Benzophenone	6
Biphenyl	3
Biphenyldiol	1
Bisphenol	27
Carbamate	3
Carboxylic acid	4
Chalconoid	6
Chlorinated aromatic hydrocarbon	1
Chlorinated bridged cycloalkene	2
Chlorinated cycloalkane	1
Chlorinated cyclodiene	4
Chlorinated phenol	4
Coumarin	1
Crown ether	1
Cyclodiene	1
Dioxin	1
Diphenolalkane	18
Diphenolalkene	4
Diphenyl ether	2
Diphenylalkane	5
Ester	1
Flavanone	10
Flavone	14
Glucuronide	1
Heterocyclic aromatic aldehyde	1
Imidazole	1
Indane	1
Indene	15
Isoflavone	15
Nitrobenzene	1
Nitrogen heterocycle	2
Organochlorine	44
Paraben	7
Phenol	40
Phenoxy carboxylic acid	1
Phosphate ester	1
Phthalate	13
Phthalimide	1
Piperidine	1
Polychlorinated biphenyl (PCB)	93
Polycyclic aromatic hydrocarbon	42
Polyether	1
Pteridine	1
Purine	1
Pyrazole	1
Pyrethrin	6
Pyrethroid	6
Resorcylic acid lactone	6
Siloxane	2
Steroid, nonphenolic	58
Steroid, phenolic	69
Stilbene	40
Sulfoxide	1
Terpene	2
Tetrahydrophenanthrene	1
Thiophene	2
Triazine	9
Triphenylethylene	49
Triphenylmethane	2
Not classified	7

Table 3-2 Product Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Product Class	Number of Substances
Analytical reagent	1
Antioxidant	1
Chemical additive	2
Chemical intermediate (adhesive, coatings, cosmetic, dye, elastomer, fiber, film, flavor, fragrance, lubricant, monomer, pesticide, plasticizer, pharmaceutical, polyester, polymer, resin, surfactant)	57
Dielectric fluid or component	54
Dye	4
Flavor	2
Food additive	16
Fragrance	6
Lubricant additive	1
Natural product (plant or animal)	31
Pesticide/Pesticide metabolite	61
Pharmaceutical/Pharmaceutical metabolite/ Pharmaceutical additive	92
Plant growth regulator	1
Plasticizer	16
Polymer	2
Preservative	5
Solvent	4
Surfactant	1
Not classified	320